A similar situation could come about in the *Festuco-Trifolietum thalii,* where *Trifolium thalii* and other Leguminosae are abundant and are intensely browsed (see Table 1). In the same seasonal period the apparent increase in calcium content can be accounted for in the same way, i.e. on the basis of the increased browsing of the leaves of the Leguminosae (Van Soest 1982). Finally, the diet presents fairly constant fibre values, at least up to the whole of August. The animals' careful grazing selection may have contributed to this phenomenon, as they choose the less fibrous parts of plants. This last aspect has been highlighted in the alpine chamois (Drescher-Kaden 1981).

Our results based on the analysis of a diet reconstructed by browsing data, need a confirm from more accurate investigations. However they suggest for the Apennine chamois what is already known in general for other ruminants (Arnold 1981): the grazing selection tends towards obtaining protein-rich diets which are however poor in fibres.

In conclusion, two results of our studies are to be emphasized: 1. In the late spring (June), summer (July-August) and early autumn (September), the females, kids and subadults of the Apennine chamois seem to depend mainly on a rare and extrazonal plant community in the Apennines, such as the *Festuco-Trifolietum thalii.*

2. The chamois grazing selection keeps this vegetation type suitable to supply a protein-rich diet in a seasonal period which corresponds to the lactation and to the early weaning of the kids.

Acknowledgements

We wish to thank the Direction of the Abruzzo National Park for providing practical facilities in the course of our research. We are also grateful to S. Lovari for the critical reading of the manuscript. The research was partly supported by a grant from the Italian Ministry of Education.

Zusammenfassung

Beschaffenheit und Qualität der Sommernahrung von Weibchen, Kitzen und Subadulten der Apenningemse, Rupicapra pyrenaica ornata Neumann, 1899 (Artiodactyla, Bovidae)

In den Monaten Juni bis September 1982–1984 wurden im oberen Val di Rose (Abruzzen-Nationalpark, Italien) an einer Herde von Apenningemsen (Weibchen, Kitze, Subadulte) monatliche Beobachtungen durchgeführt, um über die Sommernahrung Aufschluß zu erhalten. Die Vegetation im Untersuchungsgebiet oberhalb der Baumgrenze besteht aus Weiderasen *(Festuco-Trifolietum thalii).* Unsere Ergebnisse basieren auf Direktbeobachtungen der äsenden Gemsen sowie auf Analysen der aufgenommenen Pflanzen. Von Juni bis September werden ungefähr 70 % der vorhandenen Pflanzenarten abgegrast, doch die Phänologie des Weiderasens hat großen Einfluß auf die Nahrungswahl. Abschätzungen von chemischer Eigenschaft und Nährwert der Nahrung weisen darauf hin, daß das *Festuco-Trifolietum thalii* sich durch selektives Abweiden als geeignet erweist, eine proteinreiche und faserarme Nahrungsquelle während des ganzen Sommers zu liefern.

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An annual rhythm in reproductive activities and sexual maturation in male Japanese serows *(Capricornis crispus)*[1,2]

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Abstract

Changes in reproductive activities were examined in male Japanese serows captured in December to March, 1982 to 1985, in Gifu Prefecture. Examinations were conducted on the spermatogenic activity, testosterone and androstenedione levels in tissue as well as in serum and fructose concentration in the seminal vesicle. In fully adult males over 2.5 or 3 years of age, the highest values in every examination were nearly always found in December. From December to March, the values showed a decided tendency to decrease. A highly significant correlation was found, respectively, between the spermatogenic activity and the testosterone level, and between the fructose content and the testosterone level. These results indicate the presence of an annual rhythm in the reproductive activity in males of this species.

In young animals, the spermatogenic activity, testosterone levels, and fructose contents progressed increasingly with age and attained the adult levels at the age of 2.5 to 3. The concentration of androstenedione in serum of youngs showed the same level as in adults, and there was no definite tendency to decrease associated with sexual maturation.

Introduction

Until our first published study on spermatogenesis in Japanese serows (Tiba et al. 1981a, b), there had been no information available concerning reproductive physiology in males of this species, though it had been known from a few works on sexual behaviour in males, that mating occurs most frequently in October and November (Akasaka 1979; Akasaka and Maruyama 1977). As for females, several important facts had been collected about reproductive activities (Ito 1971; Komori 1975): A single young was born usually from March to June, sometimes in September but seldom in October, the first parturiton in adult females occurred usually at 3 years of age; and the gestation period was 211–213 days in captivity.

From our previous studies mentioned above, it was strongly suggested that reproductive activities in the male are subject to seasonal fluctuations; that is, in fully adult males over 2.5 or 3 years of age, the spermatogenic activity became lower from December to March of every year. It was also clarified in the previous studies that the first appearance of spermatozoa in the seminiferous tubules occurs within 6 or 7 months after birth.

The main purpose of this study is to demonstrate the presence of an annual rhythm in the testicular endocrine function in correlation with the spermatogenic activity as well as the secretory function of fructose from the seminal vesicle, which is a reliable indicator of testicular endocrine function. Another purpose is to determine exactly the completion of sexual maturation in males.

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Materials and methods

Examination items and the number of animals used are shown in Table 1. From 1982 to 1985, materials for these examinations were collected in Gifu Prefecture, which is centrally located in Japan, during the season authorized to capture the animals; namely from December to March. Animals shot in their habitats were transported to Gifu University for examination, which is situated at 50 to 150 km distance from the sites of capture. The animals had been dead usually for a few days,

Table 1. **Examination items and number of animals**

Examination items	Transported materials			Fresh materials	
	Dec. 1982 untill Mar. 1983	Dec. 1983 untill Mar. 1984	Dec. 1984 untill Mar. 1985	Dec. 1982 untill Mar. 1983	Dec. 1983 untill Mar. 1984
Testis size	150	170	181		
Seminiferous tubule diameter	31			6	7
Population of primary spermatocytes				6	7
Testosterone concentration in testes		82	123		
Testosterone concentration in serum			107		5
Androstenedione concentration in testes			126		
Androstenedione concentration in serum			109		
Fructose concentration in seminal vesicle	71	93	10		
Body weight	166	182	197	6	7
Actual number of animals examined	166	182	197	6	7

sometimes for a week or more. Most materials were obtained exclusively from the bodies within 7 days after death. Some fresh materials were acquired at the site of capture within a few hours after the animal's death.

Classification of age groups. The age estimation that underlies this study was based on the tooth eruption-wear patterns (Sugimura et al. 1981). The animals were classified into 6 age groups (Tab. 2). Animals of groups 0 to 2-I° are immature ones, in which the second dentition is not completed, and those of groups 2-I·II to 2-IV·V are adults in which all teeth are permanent.

Size of testis. The product of three dimensions of each testis was obtained, and the products for paired testes were summed.

Spermatogenic activity. The seminiferous tubule diameter was measured in one to three animals per month for every group; thus, in 15 youngs and 16 adults. The mean diameter was calculated from measurements of 10 cross sections of seminiferous tubules for each individual. Further, the number of primary spermatocytes at the pachytene stage per cross section of seminiferous tubule was counted using the fresh testicular materials removed from 13 animals (one to three animals per month in every age group except for 2-IV·V) at the sites of capture within a few hours after the animal's death. The mean number was calculated from measurements of 10 cross sections for each individual.

Testosterone and androstenedione in testicu-

Table 2. **Classification of age groups in Japanese serows**

Age group		Age in months or years
0		7–10 months
1		19–22 months
2–I°		31–34 months
2–I	2–I·II	4.6 ± 1.8 years[1]
2–II		
2–III		7.4 ± 2.7 years[1]
2–IV	2–IV·V	12.7 ± 3.9 years[1]
2–V		

[1] Calculated from the data on the patterns of cementum annulation in the teeth, which were presented by the courtesy of Dr. S. Miura

lar tissue. The testes without any pathological changes were used in this assay. Concentrations of testosterone and androstenedione were measured by radioimmunoassay using procedures similar to those described by SHODONO et al. (1975) for the determination of estradiol and progesterone in the plasma. One gram of testicular tissue was homogenized and extracted in four steps with two volumes of ether. The extract was evaporated to dryness in N_2 gas stream, re-dissolved in 1 ml of ethanol and stored at −25 °C. Before use, 0.1 ml of the stored extract was evaporated and re-dissolved in BSA-PBS to 1:300 and 1:3 for testosterone and androstenedione assay, respectively.

[1, 2, 6, 7, 16, 17-^{3}H]-testosterone (138 Ci/mmol) and [1, 2, 6, 7 ^{3}H]-androstenedione (85 Ci/mmol) were used as competitors. Each of them was added to each test assay tube as 0.1 ml solution (8,000 cpm) in BSA-PBS. The anti-testosterone-11α-succinate-BSA serum and the anti-androstenedione-3-Oxime-BSA serum each were diluted with NRS-EDTA-PBS to 1:6,000. Cross reactivity of the anti-testosterone serum with androstenedione was 2.24 %, and that of the anti-androstenedione with testosterone 5.43 %.

One-fifth ml of testicular extract, 0.2 ml of antiserum and 0.1 ml of tritiated steroid were mixed. After incubation overnight at 4 °C, the steroids, unlabeled and labeled with tritium, were reacted with dextran-charcoal. Following centrifugation at 2,500 g for 15 min, 0.8 ml of the supernatant was removed. Eleven ml of toluene scintillator containing 0.4 % PPO and 0.01 % POPOP was added, and the radioactivity was measured with a liquid scintillation spectrometer. Unknown levels of steroids were read from a standard curve and expressed as nanograms per gram of testicular tissue.

In order to investigate the possibility of a lower testicular testosterone level during the period between the animal's death and tissue sampling, a comparison was made between the materials obtained, respectively, one to two days and 4 to 5 days after the animal's death. There was no significant difference between the former (444.19 ± 96.14 ng/g; n=8, adults, Dec.) and the latter (455.27 ± 60.01 ng/g; n=8; adults, Dec.).

Testosterone and androstenedione in serum. Serum samples were obtained from the coagulated blood in the heart and stored frozen at −25 °C. Concentrations of the steroids were measured with the same method as described above. After thawing the frozen samples, 0.6 ml of serum was extracted in three steps with four volumes of ether. The extract was evaporated to dryness in N_2 gas stream and re-dissolved in 0.6 ml ethanol. Before use, 0.05 ml of the extract was decanted into another test for testosterone assay, evaporated to dryness, and diluted with 0.2 ml BSA-PBS. For androstenedione assay, 0.2 ml of the extract was treated similarly. Further procedures were the same as described for the steroids in testicular tissue. Concentrations of the steroids were expressed as nanograms per milliliter serum.

In order to examine the possibility of a lower testosterone level during the period between the animal's death and serum sampling, a comparison was made between the transported and the fresh materials (see Tab. 1). There was no significant difference between the former (3.17 ± 0.26 ng/g; n=5, adults, Jan.~Mar.) and the latter (2.94 ± 0.44 ng/g, n=5; adults, Jan.~Mar.).

Fructose concentration in seminal vesicle: After obtaining the wet weight, the seminal vesicles were stored frozen at −25 °C. But owing to the tiny size of this organ in young animals, it was extremely difficult to acquire the minimum volume of material necessary for this chemical assay. Therefore, the seminal vesicles from animals of age groups 0 and 1 were pooled so as to provide enough material. After thawing of the frozen materials, the fructose contents were measured by the method of LINDNER and MANN (1960).

To deal with the possibility of a lower fructose content during the period from the animal's death and seminal vesicle sampling, a comparison was made between the materials obtained, respectively, one and 4 days after the animal's death. There was no significant difference between the former (1.27 ± 0.43 mg/g; n=5; adults, Dec.) and the latter (1.04 ± 0.15 mg/g; n=5; adults, Dec.).

Apart from this assay, another experiment was carried out on the influence of storage upon fructose content using a bull's seminal vesicle. It was proved that the concentration remained unchanged at 4 °C for 7 days.

Body weight. As a supplementary method for determining the end of sexual maturation, the body weight was obtained in 545 animals for three consecutive seasons.

Results and statistical analysis. The mean values obtained have been shown with their standard error. Statistical comparisons between the mean values were made using Student's t test.

Results

Size of testis

The means and standard errors shown in Fig. 1 were calculated from measurement covering three consecutive capturing seasons. But before these mean values were obtained, the significance of difference in mean value between each single season was examined. No

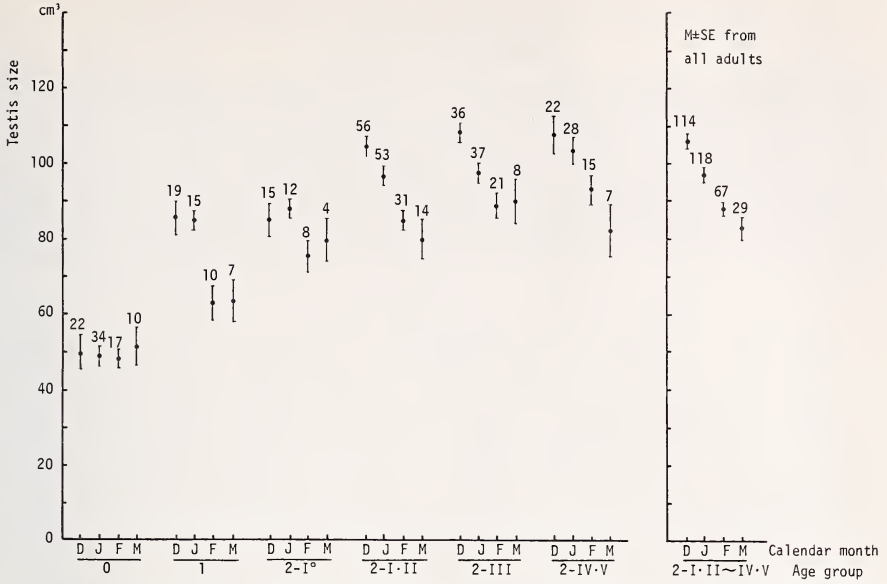

Fig. 1. Seasonal changes in combined size of paired testes for each age group of Japanese serows (M±SE, Dec. 1982–Mar. 1985). D: December; J: January; F: February; M: March. Numbers above bars represent sample size. In the following the same abbreviations are used

significant difference was found among the three seasons, and there was also no significant difference in size between paired testes.

As seen clearly in the figure, the testes grow rapidly between the age groups 0 and 2-I°, that is, from 7 months to about 2.5 years of age. The testes continue to grow slowly in the age groups 2-I·II, and thereafter the testis size is on a relatively constant level. In adults, a decrease in size occurs every year from December to March. Mean values taken from all the adults belonging to groups 2-I·II to 2-IV·V show a highly significant difference between two consecutive months ($p < 0.01$).

Testosterone concentration in testicular tissue

The first appearance of testicular testosterone is demonstrable in fawns at 7 to 10 months of age (Fig. 2). The concentration rapidly increases in age groups 0 to 2-I·II. In adults, the concentration varies widely. It is noticeable that, in age group 2-I·II, a significant increase is recognized between January and March (+196.9 %, $p < 0.05$%). A tendency to increase in the same month is found more or less in other groups except for age group 2-IV·V. But the mean value obtained from all the adults for December is significantly higher than in any other month ($p < 0.01$), and the increase from January to March is not significant.

Testosterone concentration in serum

An increase with age in the immature group is uncertain, but seasonal changes in the adults are relatively clear (Fig. 3). The mean value from all the adults in December is significantly higher than in any other months ($p < 0.01$). No significant difference was found among January, February and March.

It was also statistically clarified that there is a highly significant correlation between the concentration in serum and that in testicular tissue ($r = 0.814$, $p < 0.01$, $n = 112$).

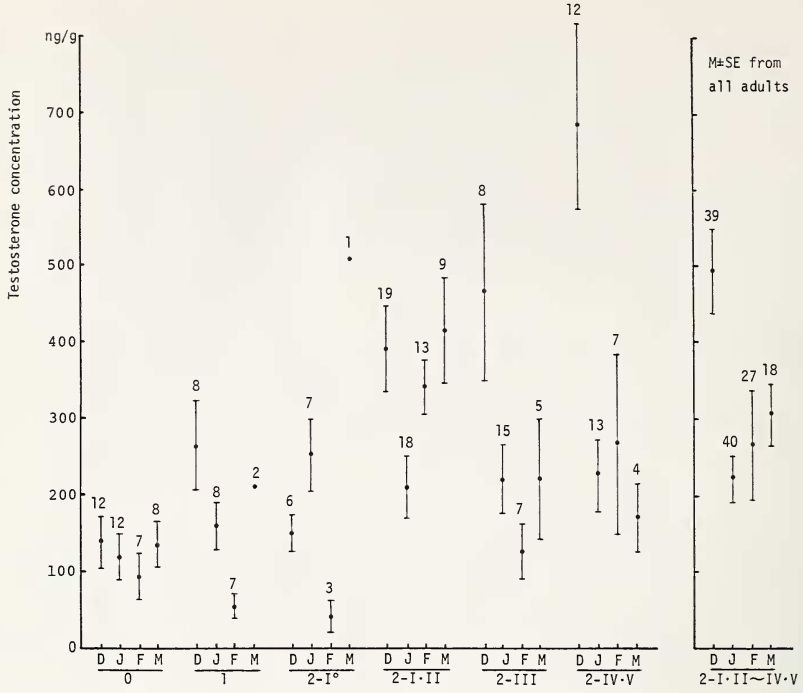

Fig. 2. Seasonal changes in testosterone concentration in testicular tissue for each age group of Japanese serows (M±SE, Dec. 1983–Mar. 1985)

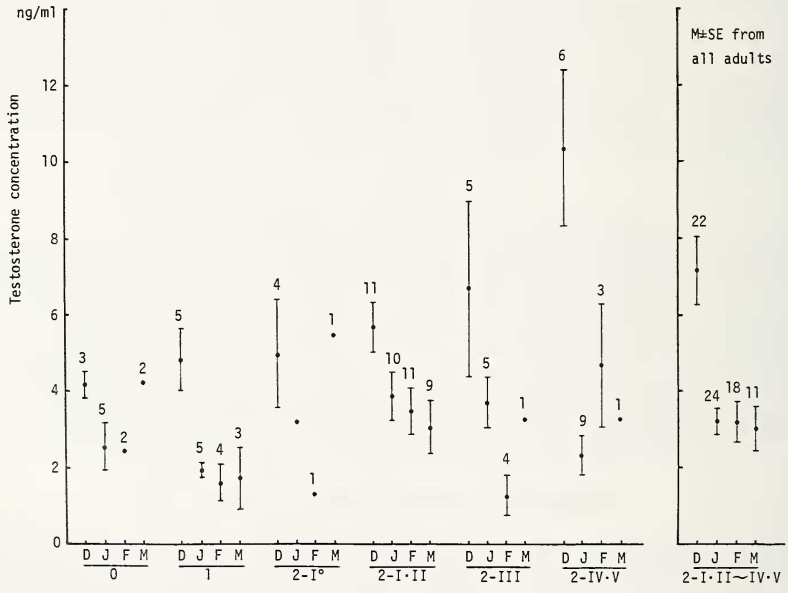

Fig. 3. Seasonal changes in testosterone concentration in serum for each age group of Japanese serows (M±SE, Dec. 1983–Mar. 1985)

Correlation between spermatogenic activity and testosterone level in testicular tissue

With the same animals in which both the seminiferous tubule diameter and the testosterone concentration in testicular tissue were measured, a correlation between the two was statistically analyzed. A highly significant correlation-coefficient was found (Fig. 4). On the other hand, in the fresh testicular materials obtained shortly after the animal's death, a highly significant correlation was found between the mean diameter of the seminiferous tubule and the mean number of the primary spermatocytes at the pachytene stage per cross section of the tubule (Fig. 5).

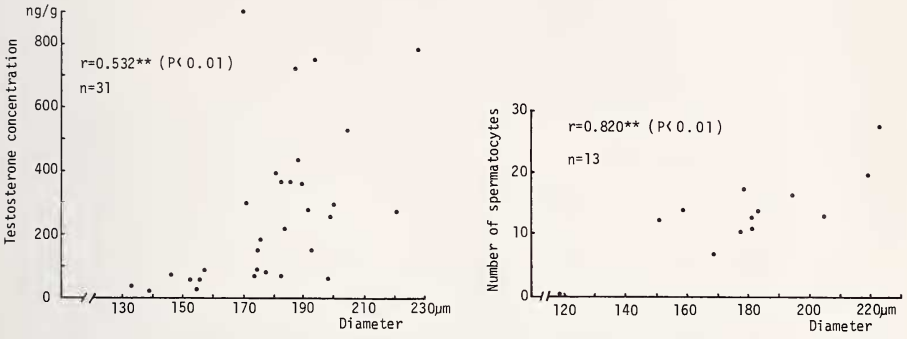

Fig. 4 (left). Correlation between seminiferous tubule diameter and testosterone concentration in testicular tissue of Japanese serows. – *Fig. 5 (right).* Correlation between seminiferous tubule diameter and number of pachytene primary spermatocytes per cross section of seminiferous tubule in Japanese serows

Androstenedione concentration in testicular tissue

In more than half of all animals examined (66/126), the androstenedione levels were under the lowest detection limit of the assay (45 pg/g). For this reason, no mean values were calculated, and the measured values were plotted for each individual in Fig. 6.

Androstenedione concentration in serum

As shown in Fig. 7, it is very difficult to find a decided tendency in the fluctuation of mean values for each age group. In the mean values calculated from all adults, the value for December is highest, showing a highly significant difference from that for February ($p < 0.01$). The second highest value in March shows a significant increase over February ($p < 0.05$), but is not significantly different from that in December.

Fructose concentration in seminal vesicle

The concentration of fructose per gram of seminal vesicle was determined (Fig. 8). For the reasons which have been advanced, however, no sufficient data for statistical analyses were obtained in the immature group, but the values obtained from young animals seem to be on a lower level than in adults. A decrease from December to March is statistically significant ($p < 0.01$) in the mean values calculated from all adults.

A statistical analysis of the correlation between fructose concentration in seminal vesicle and testosterone concentration in serum was conducted with the same animals in which both substances were evaluated. A highly significant correlation-coefficient was found between the two ($r = 0.75$; $p < 0.001$; $n = 38$).

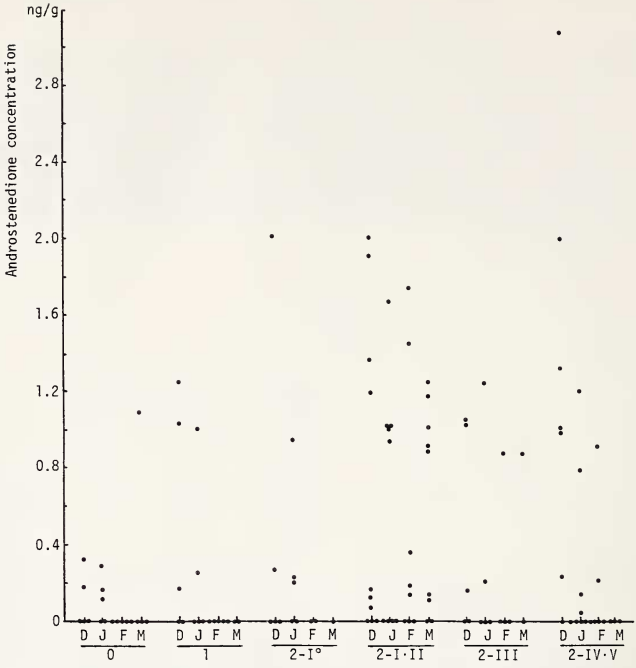

Fig. 6. Seasonal changes in androstenedione concentration in testicular tissue for each age group of Japanese serows. Spots on the abscissa indicate values below the lowest limit (45 pg/g)

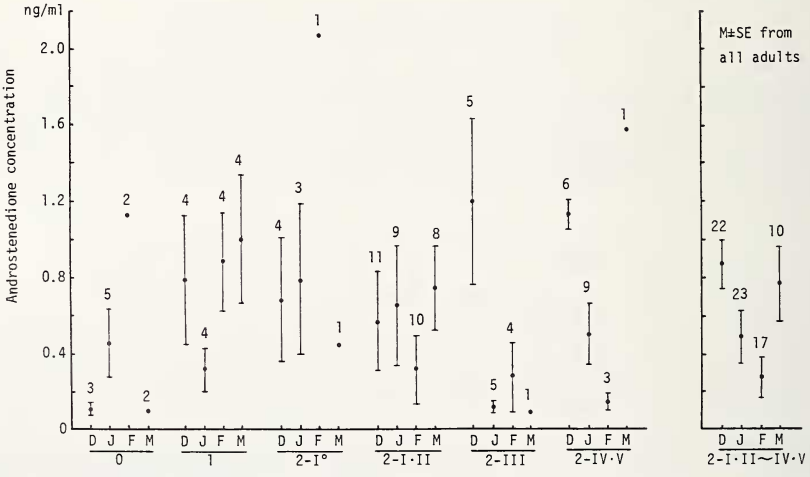

Fig. 7. Seasonal changes in androstenedione concentration in serum for each age group of Japanese serows (M±SE)

Body weight

It is clear from Fig. 9 that the growth of male animals ends at about 2.5 or 3 years of age, and thereafter the body weight is maintained on a relatively constant level. At a glance, the body weight appears to decrease steadily from December to March. However, the difference between the highest and the lowest values in each age group is not everywhere significant; thus, it is significant in group 1 (December to February: −19 %; $p < 0.01$), group 2-I·II (December to March: −9.4 %; $p < 0.01$) and group 2-III (December to February: −9.9 %; $p = 0.01$), but not in the other groups.

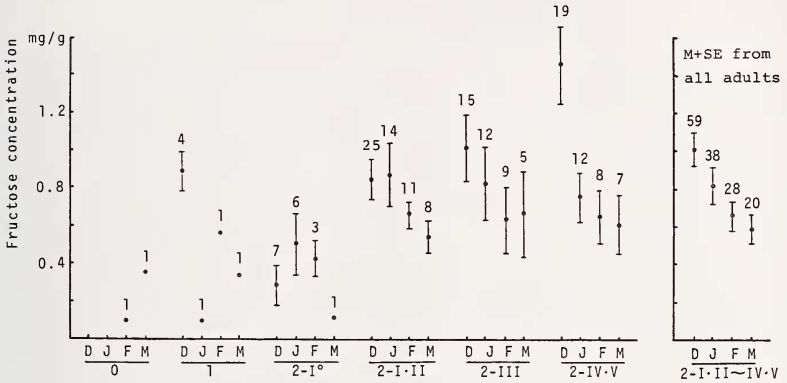

Fig. 8. Seasonal changes in fructose concentration in seminal vesicle for each age group of Japanese serows (M±SE, Dec. 1982–Mar. 1985). The values for age groups 0 and 1 were obtained from pooled material, except for the mean value for age group I in December (see text)

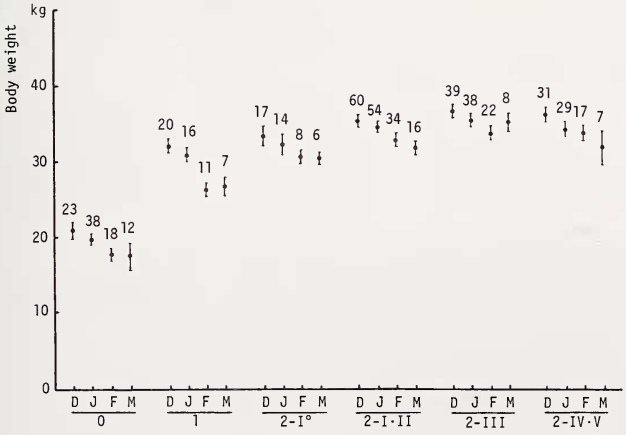

Fig. 9. Seasonal changes in body weight for each age group of Japanese serows (M±SE, Dec. 1982–Mar. 1985)

Discussion

From experiments in vitro about the metabolic routes of steroid hormone biosynthesis in the Japanese serow's testis (Nakamura and Suzuki 1985), it has been clarified that the ultimate product of androgen synthesis is testosterone in this species, too. And the major metabolic route in the testicular formation of testosterone from pregnenolone is Δ^5-pathway. It has also been demonstrated that androstenedione is produced only at a very low rate via the other minor metabolic route, Δ^4-pathway. These facts coincide with our own findings that androstenedione levels in testicular tissue as well as in serum are very low. It, therefore, can hardly be imagined that androstenedione plays an important role in the male reproductive functions of this animal. This supposition seems to be supported by our results that it was almost impossibile to find a relationship between the fluctuation in adrostenedione levels and advancing age or season.

In the goat, the domestic ruminant related most closely to the Japanese serow, the end-products in testicular formation of androgens from pregnenolone are testosterone and 17α, 20α-dihydroxy-4-pregnen-3-one; and testosterone is synthesized through both Δ^4- and Δ^5-pathways (Mori et al. 1980). As for the androstenedione levels in domestic ruminants, it is well known in the bull that the ratio androstenedione/testosterone decreases with age (Lindner and Mann 1960). Concerning changes in adrostenedione levels with advancing age in the young goat, there is a discrepancy in the literature. Leidl et al. (1970) reported, in measuring testicular androgens that there was no change in the ratio androstenedione/testosterone. On the contrary, Saumande and Rouger (1972) mentioned from the result of determination of the plasma testosterone level in one young goat of Saanen breed, that during the period of lower testicular activity androstenedione is more important than testosterone. In the ram, the testicular concentration of testosterone is always higher than that of androstenedione between the 5th and 12th month of life (Eik-Nes 1975). The sexual maturation in this animal occurs just in this period (Hulet and Shelton 1980).

Our previous indication that the testicular functions in Japanese serows are subject to seasonal fluctuation (Tiba et al. 1981a), is now clearly demonstrated by the findings that the testosterone levels in the testicular tissue as well as in serum are significantly higher in December than in any other months. It has been also clearly demonstrated in this study that the fructose content in the seminal vesicle is seasonally changeable, depending upon the testosterone level. However, we do not yet know all about the seasonal fluctuations in this animal's reproduction. The height of the breeding season is, as mentioned before, October and November, when the concentrations of steroids and fructose must be placed on even higher levels than in December.

On the contrary, the spermatogenic activity in the goat does not fluctuate in parallel with the testosterone levels in testicular tissue (Leidl et al. 1970). According to these authors, "The seasonal rhythm of reproduction in the male goat affects only the functional relationship 'androgens-accessory sexual glands-seminal plasma and contents', whereas the germinal cells show no fluctuations." And the authors add: "These findings suggest that spermatogenesis continues to be stimulated by amounts of hormones which are insufficient to stimulate the accessory sexual glands to their full function."

It is clear from the results that the sexual maturation in this animal is completed at 2.5 to 3 years of age. This coincides with the report mentioned earlier that the first parturition in female occurred usually at 3 years of age. In our own studies on morphology of ovaries and fetuses, female serows become sexually mature at about 2.5 years of age. The youngest pregnant females were 30 months old (Kita et al. 1983). If male Japanese serows reach puberty within 6 or 7 months after birth and come to full sexual maturation at 2.5 to 3 years of age, there is an interval of 2 to 2.5 years between the beginning and the end of sexual maturation. This interval appears extremely long as compared with domestic